Journal of Zoo and Wildlife Medicine 37(3): 276–280, 2006

APPLICATION OF ASSAYS FOR THE DIAGNOSIS OF TOXOPLASMOSIS IN A COLONY OF WOOLLY MONKEYS (*LAGOTHRIX LAGOTRICHA*)

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Abstract: *Toxoplasma gondii* infection is a common cause of morbidity and mortality in New World primate species. Clinical abnormalities associated with toxoplasmosis can be nonspecific, making it difficult to make a definitive antemortem diagnosis and initiate appropriate treatment. Toxoplasmosis in New World primates can have a rapid clinical course, which may lessen the diagnostic utility of antemortem tests. However, while there are a variety of *T. gondii* serum antibody tests and *T. gondii* polymerase chain reaction (PCR) assays available that are not species specific, these assays have not been comparatively applied to New World primate cases. Woolly monkeys (*Lagothrix lagotricha*), a species of New World primate, are highly susceptible to fatal toxoplasmosis. Archived serum samples from 15 living and deceased woolly monkeys housed at the Louisville Zoological Garden (Louisville, Kentucky) were tested for *T. gondii* antibodies by a commercially available latex agglutination kit, a commercially available indirect hemagglutination kit, and the modified agglutination test. In addition, aliquots of the sera were assayed for *T. gondii* DNA using a PCR assay. Both woolly monkeys that died of disseminated toxoplasmosis were positive in all four assays, indicating that each could be used to aid in the diagnosis of toxoplasmosis in this species. We suspect that these assays have applications to other species of New World primates.

Key words: Diagnostic assay, *Lagothrix lagotricha*, *Toxoplasma gondii*, toxoplasmosis, woolly monkey.

INTRODUCTION

New World primates are exquisitely sensitive to clinical infection with the coccidian parasite *Toxoplasma gondii*. Fatal toxoplasmosis has been reported in most New World primate genera, including marmosets and tamarins (*Saguinus* sp., *Leontopithecus* sp., *Callithrix* sp.),[2,11,14,21] squirrel monkeys (*Saimiri* sp.),[4,8,10,14,18,19] night monkeys (*Aotus* sp.),[4,14,21,23] spider monkeys (*Ateles* sp.),[19] uakaris (*Cacajao* sp.),[23] titi monkeys (*Callicebus* sp.),[23] saki monkeys (*Pithecia* sp.),[11] howler monkeys (*Alouatta* sp.),[14,15] capuchin monkeys (*Cebus* sp.),[20] and woolly monkeys (*Lagothrix* sp.).[5,14,15] The clinical course is typically rapid, sometimes with the animal being found dead with no preceding outward signs of illness.[14] Toxoplasmosis in New World primates can affect multiple organ systems, and clinical signs can be nonspecific or misleading, which makes a clinical diagnosis challenging.

The Louisville Zoological Garden in Louisville, Kentucky, has maintained woolly monkeys (*Lagothrix lagotricha*) in captivity since opening to the public in 1969. Cases of toxoplasmosis have affected this population sporadically, with single clinical cases in 1991, 2000, and 2002. The 1991 case presentation was characterized by sudden death. The latter two cases presented with an acute onset of illness, had multiple organ involvement, and were also ultimately fatal. Route of transmission in these cases could not be definitively determined; however, it is speculated that transmission was attributable to ingestion of tissue cysts within infected intermediate hosts, such as wild birds or rodents captured and consumed by the monkeys. The possibility also exists that sporulated *T. gondii* oocysts could have been passed into the environment by infected feral cats or could have been carried into the area by transport vectors, such as insects.

It would be optimal to avoid future cases of toxoplasmosis in New World primates by minimizing contact with potential intermediate hosts as well as with feline feces. However, since eliminating such contact in a zoo setting is extremely difficult, cases will likely continue to occur. Therefore, a routinely available, simple diagnostic procedure is needed so that clinically ill, *T. gondii*–infected animals can be promptly detected and administered appropriate treatment. Clindamycin, sulfonamides, and pyrimethamine are examples of drugs used to treat toxoplasmosis in clinically ill humans and other species.[9] In addition to diagnosing acute infections,

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T. gondii assays may also be valuable in determining toxoplasmosis infection history within a colony.

While there are a variety of non–species specific *T. gondii* antibody tests available, to our knowledge, assays have not been comparatively applied to New World primate cases. After infection by *T. gondii*, most species begin to make antibodies that are detectable within days to weeks. However, if toxoplasmosis is peracute, it is possible that serologic test results could still be negative when the animal initially becomes ill. Polymerase chain reaction (PCR) assays to amplify *T. gondii* DNA have been used with biological specimens from other mammals, including dogs and cats.[6,22,25] *Toxoplasma gondii* parasitemia, as identified by the presence of DNA in blood, may be positive before the detection of serum antibodies. The objective of this study was to assay sera from woolly monkeys with and without evidence of toxoplasmosis for *T. gondii* DNA by use of a PCR assay and for *T. gondii* antibodies by use of a commercially available latex agglutination (LA) kit, a commercially available indirect hemagglutination (IHA) kit, and the modified agglutination test (MAT).

MATERIALS AND METHODS

Archived serum samples from seven living and eight dead (15 total) woolly monkeys housed at the Louisville Zoological Garden were selected for this study. Cases were chosen based on availability of banked sera. It would have been optimal to perform the PCR assays on whole blood, because *T. gondii* can be cell associated, but those samples were unavailable. Two of the selected 15 monkeys died of disseminated toxoplasmosis that was confirmed via histopathology and immunohistochemistry (Table 1). One serum sample from each monkey was thawed, aliquoted into two separate vials, labeled, and refrozen. The banked sera used represented the most recent sample available for each monkey. In the two monkeys that died of toxoplasmosis, the serum samples used were obtained the day prior to death. At that time, the monkeys had been clinically ill for 5 days (monkey 8) and 11 days (monkey 12), respectively.

One set of 15 samples were labeled 1–15 and sent on dry ice to the Veterinary Diagnostic Laboratory at Colorado State University in Fort Collins, Colorado. Each serum sample was thawed and assayed for *T. gondii*–specific antibodies using a commercially available LA kit (Toxotest-MT; Eiken Chemical Co., San Diego, California 92111, USA) and an IHA kit (TPM-Test; Wampole Laboratories, Cranbury, New Jersey 08512, USA). Theoretically, both assays should detect all antibody classes against *T. gondii*. After antibody testing, 200 μl of each serum sample was pipetted into a 1.5-ml microcentrifuge tube and centrifuged at 16,100 g for 5 min. The supernatant was removed, the pellet was resuspended in 200 μl of 0.01 M phosphate-buffered saline, and the DNA was extracted as for the blood and whole serum (QIAamp DNA Blood Mini Kit; Qiagen, Inc., Valencia, California 91355, USA). An end-point PCR assay for the detection of *T. gondii* DNA was then performed on each digest, as previously described.[16] Samples from which the appropriate DNA product was amplified were considered positive.

A second set of 15 samples were labeled A–O and were sent on dry ice to the Animal Parasitic Disease Laboratory in Beltsville, Maryland. Each serum sample was thawed and assayed for *T. gondii*–specific antibodies using the MAT, which primarily detects immunoglobulin G antibodies.[12]

RESULTS

Sera from both monkeys that died of fulminate toxoplasmosis were positive for *T. gondii* antibodies via LA, IHA, and MAT (Table 1). Monkey 11, a clinically healthy living monkey, was positive for *T. gondii* antibodies via the LA kit but negative via IHA and MAT when first assayed. After the sample code was broken, the sample was tested again and was shown to be negative for *T. gondii* antibodies in LA and IHA. Sera from the other 12 monkeys were negative for *T. gondii* antibodies via all three serologic assays. *Toxoplasma gondii* DNA was present and was amplified from the serum of both monkeys that died of fulminate toxoplasmosis but from none of the 13 other monkeys.

DISCUSSION

On the first assay of all samples, monkey 11 had the only discordant result (LA positive, IHA negative, MAT negative, and PCR negative). When assayed again with the remaining sample aliquot, the LA results were negative. *Toxoplasma gondii* antibodies are very stable, and it is unlikely that a sample would go from strong positive to negative because of a single freeze-and-thaw cycle. Thus, we believe that the negative result is true and the initial positive result was false and resulted from operator error.

In this study, there were two monkeys with confirmed clinical toxoplasmosis, and both of these monkeys were positive in all four assays evaluated herein. If the discordant LA results are discounted, the other 13 monkeys were negative in all four assays, which indicates that they had not been exposed to *T. gondii*. Each of the assays used in this

Table 1. Background information and *Toxoplasma gondii* test results on 15 woolly monkeys from the Louisville Zoological Garden that were evaluated for toxoplasmosis via four diagnostic assays.

Monkey No.	Sex	Status	Age at sampling (yr)	Sampling to death interval	Cause of death	Assay[a]			
						LA	IHA	MAT	PCR
1	M	dead	21.5	5 mo	cardiomyopathy, hypertension, glomerulonephritis	neg	neg	neg (<1:25)	neg
2	F	dead	15	1 yr, 10 mo	undetermined (neurologic disease)	neg	neg	neg (<1:25)	neg
3	F	dead	19.5	0 days	hypertension, mesenteric thrombosis, pulmonary edema, glomerulonephropathy	neg	neg	neg (<1:25)	neg
4	F	dead	10	0 days	hepatic necrosis (woolly monkey hepatitis B virus infection)	neg	neg	neg (<1:25)	neg
5	M	living	15.5	n/a[b]	n/a	neg	neg	neg (<1:25)	neg
6	M	living	14	n/a	n/a	neg	neg	neg (<1:25)	neg
7	M	dead	13.5	1 yr, 3 mo	aortic thrombosis, cardiomyopathy	neg	neg	neg (<1:25)	neg
8	M	dead	8.5	1 day	toxoplasmosis (confirmed by IHC[c])	1 : 1024	>1:2,048	POS (>1:200)	POS
9	M	living	11.5	n/a	n/a	neg	neg	neg (<1:25)	neg
10	M	dead	10.5	0 days	disseminated inflammation (presumptive woolly monkey hepatitis B virus infection), hypertension, glomerulonephritis	neg	neg	neg (<1:25)	neg
11	M	living	11	n/a	n/a	neg[d]	neg	neg (<1:25)	neg
12	F	dead	7	1 day	toxoplasmosis (confirmed by IHC)	1:512	1:2,048	POS (>1:200)	POS
13	M	living	7.5	n/a	n/a	neg	neg	neg (<1:25)	neg
14	M	living	4.5	n/a	n/a	neg	neg	neg (<1:25)	neg
15	F	living	2.5	n/a	n/a	neg	neg	neg (<1:25)	neg

[a] LA, latex agglutination; IHA, indirect hemagglutination; MAT, modified agglutination test; PCR, polymerase chain reaction.
[b] n/a, not applicable (monkey is still living).
[c] IHC, immunohistochemistry.
[d] This sample tested false positive (1:512) initially.

study has been evaluated for analytic specificity and does not appear to detect antibodies against other protozoans (Lappin, unpubl. data). The analytical sensitivity for the assays in this species is unknown; to our knowledge, this is the first report of their use. However, the assays are thought to detect small amounts of antibodies in other species. In other species, including cats and humans, clinical toxoplasmosis can occur before the detection of *T. gondii* antibodies.[17] Thus, the diagnostic sensitivity of serologic tests, when used in cases with peracute or acute disease, can be low. In cases with suspected toxoplasmosis and negative serologic test results, a second antibody titer should be performed to assess for seroconversion. Whether or not detection of *T. gondii* DNA in blood will precede development of antibodies in this species will have to be determined in future experiments.

While additional samples from healthy woolly monkeys and woolly monkeys with clinical toxoplasmosis will be needed to allow for accurate determination of sensitivity, specificity, positive predictive value, and negative predictive value, these results indicate that all four assays can be used with serum from woolly monkeys. Both the LA and IHA can be performed as point-of-care assays; New World monkey care providers should consider having one of these tests on site for evaluation of clinically ill monkeys and to track serologic evidence of exposure. Because the antibody tests were all positive in the woolly monkeys with clinical toxoplasmosis, there appears to be no obvious benefit to performing the more expensive and technically difficult PCR assay. However, blood, serum, and other body fluids or tissues from affected monkeys could be saved frozen and assessed by PCR assay at a later date.

The two woolly monkeys with clinical toxoplasmosis had been ill for short periods of time (5 and 11 days, respectively). These results indicate that the monkeys were acutely ill but were able to mount a detectable antibody response very quickly. Alternately, the monkeys could have been chronically infected with *T. gondii* and developed reactivation of chronic infection leading to clinical illness. Recrudescence of clinical toxoplasmosis from activation of tissue cysts is known to occur in other species.[1,3,7] With the data available, we cannot definitively determine whether the *T. gondii* infections were acute or chronic. Pathogenicity of *T. gondii* in New World primates is thought to primarily be determined by the pathogenicity of the strain and the exposure dose. However, stress and other immune suppressive states may also be contributory factors with regard to how a host is affected by *T. gondii* infection.[13]

Toxoplasma gondii infections in New World primates are not always fatal, as there are reports of monkeys suffering transient illness but surviving epidemics.[8] Additionally, serologic surveys of wild squirrel monkeys in Brazil revealed that 24 of 49 individuals tested positive in the IHA test to *T. gondii*.[10] Failure to detect *T. gondii* antibodies or DNA in the 13 unaffected woolly monkeys described here indicates that the monkeys had not been exposed to the organism. This result was unexpected, since other members of this colony have succumbed to toxoplasmosis, monkeys are continually exposed to wild birds and other potential intermediate hosts, and woolly monkeys are particularly proficient at bird predation.[24]

Acknowledgments: We thank Elizabeth Hayden, Melissa Brewer, and Jennifer Hawley for their technical assistance; Dr. James Raymond for his pathology services with regard to the two monkeys that died of toxoplasmosis; and Candy McMahan, Silvia Zirkelbach, and the other members of the woolly monkey keeper staff at the Louisville Zoo for their support and dedication.

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Received for publication 22 February 2005